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SHORT COMMUNICATION

Sex differences in early instar behavior in *Pholcus phalangioides* (Araneae: Pholcidae)

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Abstract. Intersexual differences in juvenile behavior in invertebrates are poorly understood despite the recognition that they may be widespread. We present a study designed to explore sex differences in behavior in early instar long-bodied cellar spiders *Pholcus phalangioides* (Fuesslin 1775). Our findings reveal that sex differences in activity and feeding are present early in *P. phalangioides*, which may have important implications for studies of behavior that involve juveniles. Further investigation of the factors that underlie the early emergence of sex differences in behavior is needed.

Keywords: Juvenile, sex difference, behavior, cellar spider

Sex differences in adult behavior have been widely studied and are often ascribed to factors related to sexual selection including anisogamy (Bateman 1948), inequities in parental investment (Trivers 1972), and operational sex ratio (Emlen & Oring 1977) (Davies 1991; Andersson 1994). Significantly less is known about juvenile sex differences in behavior, despite recognition that they are likely to be prevalent for many reasons. For example, juvenile sex differences in behavior may emerge as consequences of sex-specific relationships among growth, survival, and fitness (Johnsson et al. 2001), as side-effects of sexually selected gene activity that promotes sexual differences in adults (Cheverud et al. 1983; Bakker 1996), or developmental programs that produce gradual changes in behavior instead of abrupt changes during sexual maturation (Williams 1992). Mammals and birds have received the greatest attention in studies of juvenile sex differences in behavior, and most of these studies have concentrated on play fighting (e.g., Eaton et al. 1986; Biben 1998; Paukner & Suomi 2008; Raihani et al. 2008). Only scarce attention has been paid to invertebrates in studies of juvenile sex differences in behavior (e.g., Singer & Riechert 1994; Persons 1999), and results from these few investigations are sometimes difficult to interpret. For example, Stevens et al. (2006) discovered sex differences in juvenile dispersal in corophiid amphipods, however they could not ascertain if sex differences were behavioral per se or artifacts of other factors.

We report an investigation exploring behavioral sex differences in early instar long-bodied cellar spiders *Pholcus phalangioides* (Fuesslin 1775). *P. phalangioides* is a cosmopolitan species strongly associated with human habitation. Hatched juveniles develop into adults after approximately 100 days and five molts (Uhl et al. 2004). Sexual dimorphism in this species is minimal, and in some populations, males are slightly larger than females (Uhl 1994). In the current investigation, we quantified the amount of time that early instar juveniles spent active in novel environments as well as the number of prey they consumed in a 24-h period. Once spiders matured and sex could be unambiguously assigned, we determined if there were sex differences in their juvenile behaviors. Voucher specimens were deposited in the Arcadia University insect collection.

In our investigation, we used juveniles that were second or third instar offspring of laboratory-raised adults that we originally collected from Pearson Hall at Miami University (Oxford, Butler County, Ohio, USA). We separated juveniles from their mothers after they molted to the second instar and maintained them individually in 5.5 cm high × 5.5 cm diameter clear, cylindrical, plastic containers. We fed juveniles two vestigial-winged *Drosophila melanogaster* once a week and housed them in a laboratory at Arcadia University (Glenside, Montgomery County, Pennsylvania, USA) with a relative humidity ~ 60%, a photoperiod of 12L:12D, and a temperature of approximately 25° C. Following the conclusion of all test trials, we raised juveniles to adulthood, switching their diets from *D. melanogaster* to two domestic crickets (2–6.35 mm; *Acheta domesticus*) once a week. Following maturation, we sexed all spiders.

We randomly assigned 59 juveniles (mean days post-molt = 4 days ± 0.82 SD) to one of two treatment groups: activity or foraging. We placed juveniles assigned to the activity group (n = 29) individually into unused plastic containers (described above) for a period of 15 min. Using stopwatches during live trials, we recorded the amount of time that each juvenile spent active during this period. An "active" spider was a spider moving any part of its body or its entire body. We did not discriminate between different types of activities (e.g., walking, web-building, etc.). Because the amount of time that juveniles spent active was not normally distributed, we made statistical comparisons between the sexes using a nonparametric Mann-Whitney U Test.

We did not offer juveniles assigned to the foraging group (n = 30) prey 24 h before trials commenced. To conduct a trial, we placed 10 vestigial-winged *D. melanogaster* with a juvenile spider in its home enclosure, and we recorded the number of flies that were consumed after a period of 24 h. Following sex assignment, we compared the number of flies consumed by the sexes using a two-sample t-test. We used JMP^{TM} 5.1.2 statistical software to conduct all analyses.

We discovered statistically significant sex differences in both activity and foraging in early instar *P. phalangioides*. Juvenile males were significantly more active in enclosures than females (Mann-Whitney U Test, $Z = 3.68$, $P = 0.0002$, $n = 29$, Fig. 1), and juvenile females consumed significantly more flies in a 24 hr period than males (t ratio = 2.96, $P = 0.007$, $n = 30$, Fig. 2).

The results of our study suggest that sex differences in activity and feeding emerge early in *P. phalangioides*, and to our knowledge, this is a very rare documentation of juvenile sex differences in behavior in an invertebrate and in a spider. Our results have important implications for behavioral studies of spiders and perhaps other arthropods that include juveniles, as biased offspring sex ratios may confound the interpretation of results. For example, female biased sex ratios are common in social spiders, which have precise control of offspring sex ratio (Avilés 1986, 1993; Avilés et al. 1999, 2000). One species of solitary spider has been reported to have a female-biased sex ratio (Gunnarsson & Andersson 1992; Gunnarsson et al. 2004), and we

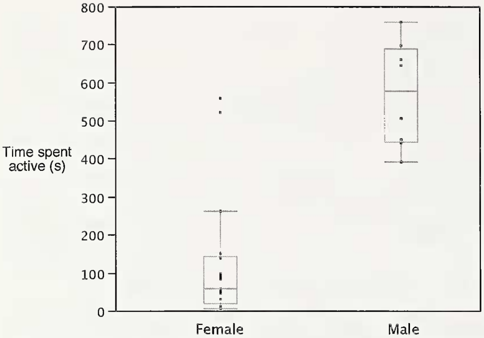

Figure 1.—The amount of time that early instar male (n = 8) and female (n = 21) *P. phalangioides* spent active in plastic enclosures. Box plots show lower and upper quartiles, and lines across boxes identify the median sample value.

Figure 2.—The mean number of flies consumed ± SE by juvenile male (n = 11) and female (n = 19) *P. phalangioides*.

have discovered female-biased, offspring sex ratios in *P. phalangioides* (Rypstra & Hoefler unpubl. data). For behavioral studies of species such as these and others, behaviors unique to the majority sex will be overrepresented in analyses if juvenile sex is not considered.

The reasons why *P. phalangioides* exhibit sex differences in juvenile behavior is not presently known, however several factors may be wholly or partially responsible. One possible explanation is that the intersexual differences discovered in our study are a result of an early divergence in developmental programs for males and females, whieh gradually prepares them for different roles during the reproductive phase of the life cycle. Historically, behavioral differences attributed to sex in spiders include active male dispersers that feed infrequently and sedentary females that feed often (Foelix 1996). Because *P. phalangioides* do not disperse by ballooning (Schäfer et al. 2001), the higher activity of males may be a reflection of male-biased dispersal and a result of selection favoring inbreeding avoidance (Bonte 2009). Similarly, through scramble competition, sexual seleetion may favor adult males that are able to locate and fertilize multiple females quickly (Andersson 1994), and this quality of high activity may develop early and gradually. The greater incidence of feeding by juvenile females may be a result from a correlated response to fecundity selection promoting higher feeding rates in adult females (Fox & Czesak 2000). To gain a clearer understanding of these possible underlying explanations, future studies should consider intersexual differences during successive ontogenetic stages.

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